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Response of benthic metabolism and nutrient cycling to reductions in wastewater loading to Boston Harbor, USA

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ABSTRACT

We describe the long-term response of benthic metabolism in depositional sediments of Boston Harbor, MA, to large reductions in organic matter and nutrient loading. Although Boston Harbor received very high loadings of nutrients and solids it differs from many eutrophic estuaries in that severe hypoxia was prevented by strong tidal flushing. Our study was conducted for 9 years during which a series of improvements to sewage treatment were implemented, followed by 10 years after the culminating step in the clean-up, which was to divert all wastewater effluent offshore. Counter to expectations, sediment oxygen demand and nutrient effluxes initially increased at some stations, reaching some of the highest rates recorded in the literature, and were spatially and temporally quite variable. Early increases were attributed to macrofaunal effects, as sediments at some sites were rapidly colonized by tube-building amphipods, *Ampelisca* spp., which dominated a dense macrofaunal mat community. As reductions in loading progressed, however, mean rates in oxygen uptake and release of ammonium, nitrate, and phosphate all decreased. At the point of outfall diversion, rates and variability had already decreased substantially. By the end of the study, average oxygen uptake had decreased from 74 to 41 mmol m^{-2} d^{-1} and spatial and temporal variability had decreased. Similarly, nutrient fluxes were less than half the rates measured at the start of the project and also less variable. Other evidence of improved conditions included a decrease in the carbon content of sediments at most stations and higher Eh values at all stations, illustrating less reducing conditions. Denitrification also showed an overall decrease from the beginning to the end of the 19-year study, but was highest during the intermediate phases of the cleanup, reaching 9 mmol N m^{-2} d^{-1}. At the end of the study denitrification averaged for all sites was 2.2 mmol N m^{-2} d^{-1}, but when compared to current loadings, had become a more important overall sink for N within the harbor. Few long-term examinations of the responses of sediment biogeochemistry to reductions in nutrient and organic matter loading have been reported. Our findings demonstrate that benthic fluxes may respond to reductions in loading in complex ways, and sediments need not represent a long-term legacy that would impede ecosystems recovery.

1. Introduction

Excessive anthropogenic inputs of nutrients and labile organic matter and consequent eutrophication of estuaries have led to declining water quality, hypoxia, and blooms of undesirable algae (Nixon, 1995; Conley et al., 2009; Duarte, 2009). Concern over degrading conditions and concomitant loss of ecosystems services has led to a number of attempts to reduce the amount of these nutrients entering eutrophic estuaries. There is a great deal of interest in evaluating how successful these attempts have been and in learning how to predict the trajectory of eutrophication reversal or "oligotrophication" (Duarte, 2009; Nixon, 2009). Studies to date (Duarte et al., 2009; Taylor et al., 2011) have shown that not all estuaries respond similarly to decreases in nutrient loading, and that some systems do not revert to their original conditions.

Sediments play an important role in organic matter decomposition and nutrient cycling (Nixon, 1981; Boynton and Kemp, 1985) and may impact the rate at which estuaries respond to changes in

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nutrient loading. Some have argued that sediments can greatly slow ecosystem recovery because they contain substantial stocks of organic matter and nutrients (Soetaert and Middelburg, 2009). Others have pointed out that the labile portion of sediment organic matter should be quickly depleted and sediments should respond rather rapidly to changes in loading (Kelly et al., 1985; DiToro, 2001). Few investigations have directly addressed the biogeochemical response of sediments undergoing nutrient reductions at the ecosystem scale (Borja et al., 2006; Boynton et al., 2008).

Boston Harbor, Massachusetts (Fig. 1) is an urban estuary that historically received large inputs of nutrients and suspended solids. Poorly treated wastes were delivered through outfalls within the harbor as sewage plant effluent and as sludge/effluent mixtures. Wastewater was also delivered directly through numerous combined sewer overflows (CSOs), which, in older systems like Boston's where storm water and wastewater are transported through the same pipes, are activated to prevent backflow when very heavy rainfalls overwhelm the capacity of the treatment system. Several rivers within the greater Boston metropolitan area also empty into the harbor, delivering suburban runoff and industrial wastes. Although both northern and southern sections of the harbor have suffered from long-term wastewater inputs, water and sediment quality in some areas were more severely affected than in others, depending on proximity to these sources and to local tidal flushing (Kelly, 1997). It is important to note that despite very high nutrient loading, Boston Harbor did not experience some symptoms of hyper-eutrophication, such as nuisance algal blooms and hypoxia (Taylor et al., 2011) common to other nutrient-impacted estuaries because it is relatively shallow and has a short average water residence time (Stolzenbach and Adams, 1998). This allowed investigation of benthic responses to large changes in organic matter and nutrient loading separate from the usual accompanying

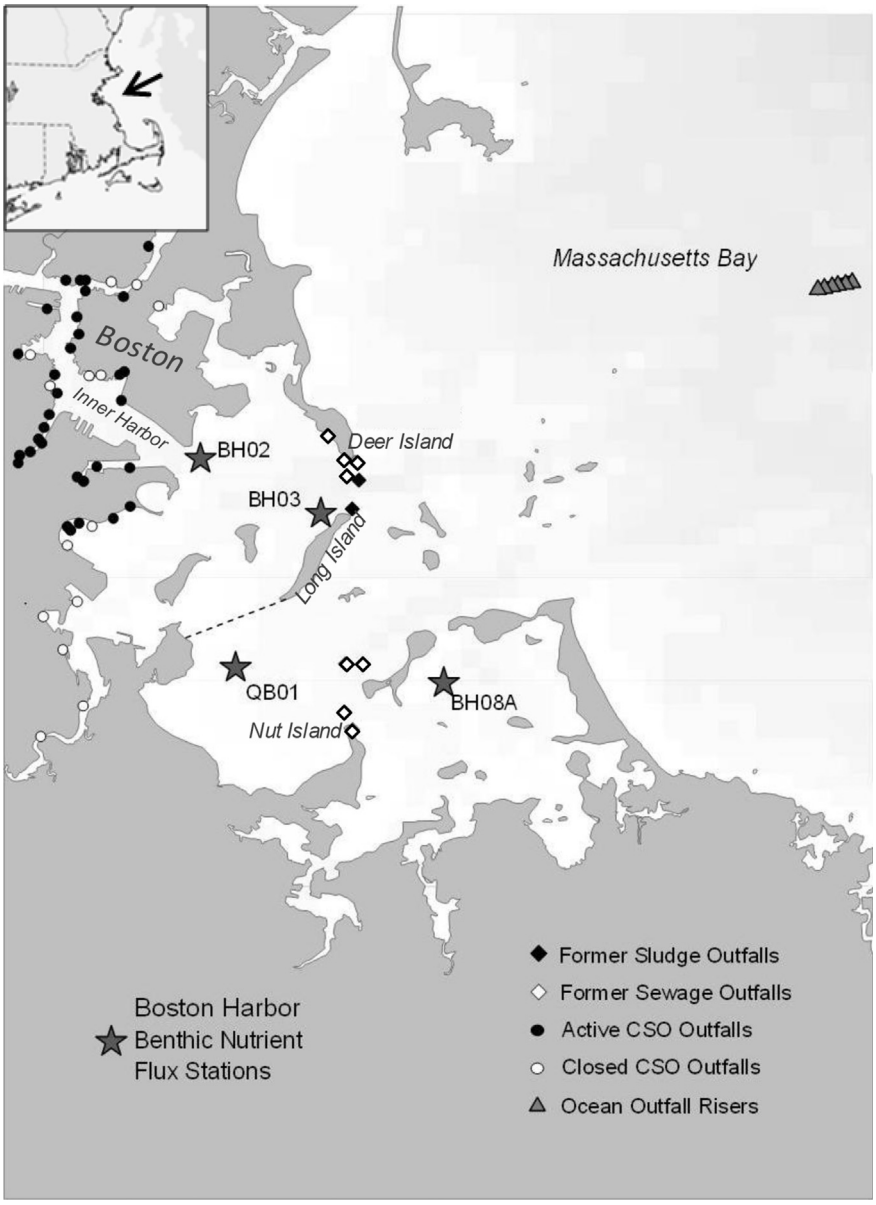

Fig. 1. Map of Boston Harbor, showing Stations BH02 and BH03 in the northern harbor, and Stations QB01 and BH08A in the southern harbor, with the two regions divided by the dotted line between the mainland and Long Island. Also shown are Deer Island and Nut Island (sites of wastewater treatment plants), locations of former outfalls, locations of closed and active CSOs, and the location of the ocean outfall, 9 miles offshore in Massachusetts Bay. Inset shows location of Boston Harbor along the Massachusetts coast, northeastern USA.

conditions of bottom water oxygen stress, which tend to exacerbate the effects of high carbon loading in sediments (Conley et al., 2009).

To reduce these inputs and address the suite of issues associated with the harbor's growing eutrophication, the Massachusetts Water Resources Authority (MWRA) began a comprehensive upgrade to wastewater treatment facilities and practices in 1991. Improvements progressed in stages, which resulted in step changes in many aspects of loading, especially for particle-associated components. The sequence of upgrades has been defined as four treatment periods by Taylor (2010) and adapted here as described in Table 1. Period I may be considered the "pre-cleanup" period. The end of this period, December 1991, was marked by the cessation of sludge disposal within the harbor, the first major milestone of the project. The result was a reduction of total suspended solids (TSS) loading to the harbor from the treatment facilities by over 35%, from over 95 to about 60 metric tons(t) day^{-1} (Fig. 2a, modified from Taylor, 2010). This level of loading was experienced by the harbor until mid-Period II (1995), when further reductions were accomplished with the completion of a new primary treatment plant at the Deer Island Treatment Facility in the northern harbor. In primary treatment, about 60% of solids are removed from the waste stream in settling chambers. Secondary treatment was begun and fully implemented at Deer Island during the last two years of Period II (1997–1998). After secondary treatment, in which oxygen is added to enhance microbial activity, 80–90% of solids are removed, so during Period III, further reductions in loading were realized. Also in Period III, wastewater flows from the southern harbor treatment facility at Nut Island were transferred to the Deer Island plant, such that flows were consolidated and all received secondary treatment before discharge through the Deer Island outfall. By the end of Period III, solids loading to the harbor had dropped by almost 90% to about 12 t d^{-1}. In contrast, decreases in dissolved inorganic nitrogen (DIN) loading were small because secondary treatment does not remove DIN. Consequently, reductions in total nitrogen (TN) loading lagged behind those for TSS (Taylor, 2010).

Period IV, the final phase of the project, began in September 2000, when all sewage effluent was diverted offshore to a deep-water outfall in Massachusetts Bay. With the ocean outfall operational, effluent loading of TSS to the harbor was all but eliminated. In addition, there was an 82% reduction in total nitrogen (TN) loading and a decrease in DIN concentrations in the harbor water column of over 50% (Taylor, 2010). For the benthic environment, the most important result of these successive improvements was an overall 87% reduction in TSS loading and a 50% reduction in primary production from the initiation of the project to Period IV.

As loadings to the harbor decreased, large changes occurred in the benthic infaunal community that had important impacts on sediment biogeochemistry. These changes were documented by Diaz et al. (2008; see Table 1 and Fig. 2b) who used sediment profile imaging (SPI) to create a chronology of change in biological and physical characteristics of sediments at stations representing a range of habitat types throughout the harbor. We have used their findings to inform our results. Their data showed that after sludge disposal ceased, the harbor was colonized by tube-building amphipods, *Ampelisca* spp., which in very high abundance form dense mats of tubes. Colonization of the harbor by *Ampelisca* spp. at mat densities (50,000–300,000 individuals m^{-2}) peaked toward the end of Period II, then steadily declined through Periods III and into IV. Late in Period IV (2007–2008), populations of another amphipod, *Leptocheirus pinguis*, increased in parts of the harbor and at about the same time, the *Ampelisca* spp. mats began to return (Maciolek et al., 2011).

In this paper we examine sediment responses during 9 years of loading reductions and 10 years under the new loading regime. We measured benthic metabolism, nutrient cycling, and sediment properties to determine: 1) how rapidly benthic respiration, nutrient regeneration and denitrification responded to changes in loading; 2) how quickly, if at all, sediment properties such as redox and % carbon changed; and 3) whether the role of the benthos in carbon and nutrient cycling in the harbor as a whole changed as external loads were reduced.

2. Materials and methods

2.1. Site description

Boston Harbor is a tidally dominated coastal embayment within the larger Massachusetts Bay system. It is roughly divided into

Table 1
History of treatment changes by years and period (modified from Taylor, 2010), and of concurrent changes in amphipod colonization of harbor sediments. Mat coverage refers to the percent of survey stations at which *Ampelisca* spp. mats were present (Diaz et al., 2008). Note that this paper does not include data from Period I. (DI = Deer Island Wastewater Treatment Facility; NI = Nut Island Wastewater Treatment Facility; CSO = combined sewer overflow).

Period	Years	Treatment	Description	Amphipod mat distribution
I	1990–1991	Pre-Boston Harbor Project	Partial primary treatment of effluent; Effluent discharge from DI (N. harbor) and NI (S. harbor); Sludge disposal within north harbor; 46 CSOs in operation	*Ampelisca* spp. sparsely distributed
IIA	1992–1994	Post-sludge	Sludge disposal ceased (Dec. 1991); Initiate upgrade to full primary treatment at DI	*Ampelisca* spp. "bloom" begins; mat coverage increases from 5% to 61%
IIB	1995–1997	Full primary	Full primary at DI (July 1995) Initiate upgrade to secondary at DI (July 1997) 7 CSOs eliminated.	Peak period of mat coverage in 1996: mats 64%
III	1998–2000	Full secondary	Full secondary (March 1998); NI to DI transfer (April 1998) resulting in discharge from DI only; 2 CSOs eliminated; Offshore diversion (September 2000)	Mats declining; in 2000, mat coverage averages 33%
IV	2001–2010	Offshore diversion	All effluent discharged offshore; Elimination or decreased discharge from 23 CSOs, many in Lower Charles-Upper Inner Harbor in 2010; reduction in discharge volume from CSOs 90%.	Mat coverage shrinks; is 0% in 2005; *Leptocheirus pinguis* colonization and *Ampelsica* spp. mats reappear 2007–2008; in 2010 mat coverage 26%

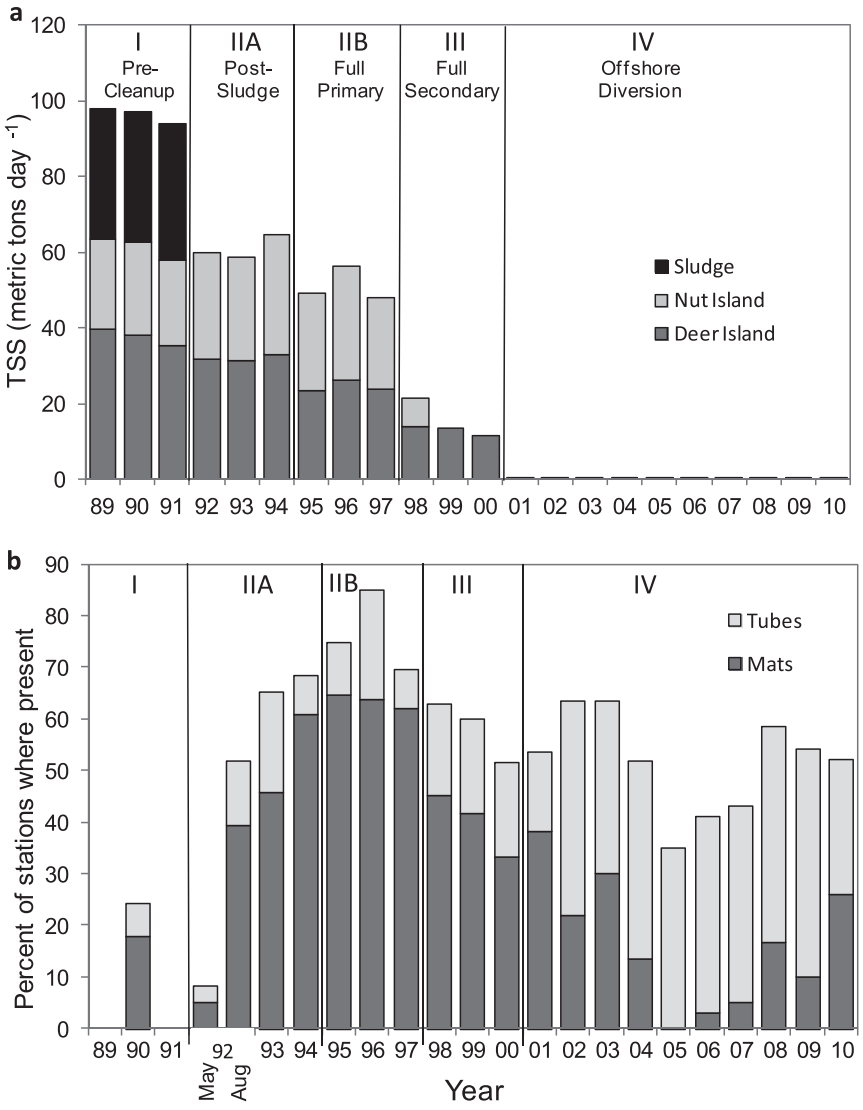

Fig. 2. (a) Total suspended solids (TSS) loading, including sludge, in MWRA treatment plant discharges, 1989–2010, by period (Werme et al., 2010), showing contribution of sludge as well as discharges from the Nut Island and Deer Island treatment plants; (b) Percent of stations sampled by SPI showing presence of *Ampelisca* spp. mats or tubes (Diaz et al., 2008 and pers. com.) by year and treatment periods. Total number of stations in 1990: 34; 1992: 11; 1993–1994: 49; 1995–2003: 60; 2004–2010: 61. Two data bars are shown for 1992 to illustrate the rapid change between May and August in this first year after sludge discharge ceased.

northern and southern portions by Long Island (Fig. 1). The ~3 m tide range and multiple inlets result in short water residence times, ranging from about 2 days near the inlets to about 17 days in some areas around the periphery of the harbor where tidal currents are weaker (Signell and Butman, 1992). Active tidal flushing produces a well-mixed water column (Kelly, 1997).

Four sampling stations were chosen to represent a range of pre-cleanup exposure to inputs, recognizing that exposure was determined not only by proximity to the Deer Island (north) and Nut Island (south) Treatment Facility outfalls, but by effluent plumes created and moved by tidal currents. Stations BH02 and BH03 were located in the northern harbor and BH08A and QB01 were in two embayments of the southern harbor. Water depths at each station varied with tide stage but on average were about 10 m for BH02, 8 m for BH03 and BH08A, and 5 m for QB01.

Modeling of effluent plume dilutions (model by Signell, as shown in Rex et al., 2002; http://www.mwra.state.ma.us/harbor/enquad/pdf/2002-09.pdf) showed that Station BH02 was under a "hot spot" of the Deer Island outfall plume and was thus strongly influenced by this outfall as long as it was in use. This site was also near the entrance to the Inner Harbor and the Charles and Mystic Rivers, whose watersheds encompass most of urban Boston and where most CSOs were located (Fig. 1). Of the four sites, only BH02 was located in a sediment reworking environment, described as having mixed grain sizes resulting from a combination of erosion and deposition, and indicative of highly variable currents (Knebel and Circe, 1995). Station BH03 was near the mouth of the harbor, across the tidal/shipping channel from the Deer Island Treatment Facility and the north harbor outfall. Although this area experiences rapid tidal flushing of the water column, the station was in a depositional area of fine-grained sediments (Knebel and Circe, 1995) near the site of sludge disposal, and was heavily impacted by these deposits.

In the southern harbor, Station BH08A was in a depositional area of Hingham Bay. Tidal circulation carried the Nut Island Treatment Facility effluent plume to this site (Rex et al., 2002). Station QB01

was in a depositional area of Quincy Bay, in an area with much higher dilution of effluent than the other three stations and considered the least impacted site. It had sediments somewhat sandier than the other three stations.

Throughout the sampling periods bottom water temperatures for the May–October period ranged from 8° to 20 °C, with coolest temperatures in May and warmest in August. Salinity ranged from 27.4 to 33.6. Lowest bottom water oxygen encountered during sampling was 6.0 mg L^{-1}, recorded at Station QB01 in August 2008.

Details of all work conducted under the monitoring program may be found in annual reports to the MWRA (http://www.mwra.state.ma.us/harbor/enquad).

2.2. Sample collection

Sampling began in 1992 at stations in the northern harbor (Giblin et al., 1997) and in 1995 at the stations in the southern harbor. Samples were collected in May, July, August, and October. At each site, two to four 15-cm diameter sediment cores were collected intact by SCUBA divers for metabolism and nutrient flux measurements. Additional 6.5-cm diameter cores were collected for Eh, pH, and porewater analyses, and 2.5-cm diameter cores were collected for chlorophyll and solid phase analyses. During some years, separate cores were collected for denitrification measurements (see details in Section 2.4). Cores were held in the dark at near-ambient temperature (±2 °C) while on deck, during transport, and during incubations.

2.3. Measurement of benthic respiration and nutrient flux

A very brief description of the protocols used for flux measurements is given here, as all procedures and protocols have been described in detail elsewhere [(see Giblin et al., 1997 and several Combined Work/Quality Assurance Project Plans (CW/QAPP), e.g., Tucker and Giblin, 2010: http://www.mwra.state.ma.us/harbor/enquad/pdf/2010-05.pdf)]. Sediment cores for flux measurements were sealed with a headspace of filtered site water and incubated in the dark at ambient temperatures (±2 °C). Dissolved oxygen concentrations in headspace water, measured by electrode, were monitored during the incubations and used to determine the duration of the incubation. Water samples for nutrients were withdrawn from the headspaces of cores through syringe ports at 4–5 timepoints while simultaneously being replaced with site water. Fluxes were calculated as the change in constituent concentration over time. Standard analytical methods for measurements other than for denitrification are summarized in Table 2. Denitrification was measured at the University of Rhode Island from 1992 to 1994 (Nowicki et al., 1997), at the University of Massachusetts (Howes) from 1995 to 1997, and at the Marine Biological Laboratory after 1997.

Table 2
Summary of measurements, analytical methods, and references.

Measurement	Method	Reference
Fluxes	Whole core, dark incubations at ambient temperature	Tucker and Giblin, 2010; Cibik and Howes, 1995
Dissolved oxygen	Polaragraphic or luminescent DO electrode	Hale, 1980; Hach, 2006
NH_4^+	Phenol-hypochlorite	Solorzano, 1969
$NO_3^- + NO_2^-$	Cadmium reduction	Diamond, 1994
PO_4^-	Molybdate-antimonyl tartrate	Murphy and Riley, 1962
Si	Molybdate-stannous chloride	Armstrong, 1951; Alpkem, 1986
Eh	Platinum electrode	Bohn, 1971
HS^-	colorimetric	Cline, 1969
TOC and TN	Perkin Elmer CHN elemental analyzer, after acidification	Zimmerman et al., 1997

2.4. Measurement of sediment denitrification

Two methods were used to measure denitrification. From 1992 through 2003, a gas chromatography (GC) method was used to measure gaseous nitrogen (N_2) in the headspace overlying paired oxic and anoxic sediment cores (Nowicki et al., 1997). After lowering background N_2 concentrations by flushing core headspaces with either He (anoxic) or He + O_2 (oxic) for 36 h, N_2 production was measured. The anoxic cores served as a "control" to account for passive N_2 diffusion from porewater. Detailed descriptions of sampling and measurement methods using gas chromatography are given in Nowicki et al. (1997), Tucker and Giblin (1998), and Hamersley and Howes (2005). While this method was in use, measurements were made at Stations BH02 and BH03 only.

In 2004, the method was changed to the N_2/argon technique (Kana et al., 1998). This method uses a quadrupole mass spectrometer equipped with a membrane inlet (membrane inlet mass spectrometer or MIMS) to precisely measure N_2/Ar ratios of dissolved gases in water samples. This method offered higher precision for gas measurements and did not require alteration of ambient conditions in the experimental cores. In addition, because the samples for N_2/Ar could be taken from the same cores that were used for flux measurements (i.e. did not require additional core incubations), denitrification measurements were expanded to all four stations.

The GC and MIMS techniques were compared during 1999 and 2003, prior to switching methods. In 1999, the GC method was performed at MBL and samples for MIMS analysis were sent to University of Maryland Center for Environmental Science. In 2003, both analyses were conducted at MBL. We tested whether overall trends could have been influenced by the change in methods. We evaluated the similarity of the two methods using a percentage similarity model (Scott et al., 2003). The two methods had a mean similarity of 86.6% (S.D. 20.9%); the similarity <100% indicates that the MIMS results were on average about 14% lower than the GC results. Although the change in methods may have contributed to an observed decrease in rates of denitrification during 2004–2007 (mid Period IV), the temporal pattern of change was well established before the method change (see supplemental materials, Fig. S1), and corresponded to the decline observed in complementary SOD and nutrient fluxes (Fig. S2). We also did observe higher rates again late in Period IV, suggesting that the MIMS technique did not miss high values. Given the large variability inherent in sediment flux measurements (typically ±20% due to substrate heterogeneity alone), it did not appear that the change in methods compromised our ability to examine major long term trends.

2.5. Infaunal assessments

Harborwide quantitative infaunal assessments were made by Diaz et al. (2008; Fig. 2b) but they did not always correspond to our sampling times or locations. We made qualitative assessments of whether amphipods, amphipod mats, or other macrofauna were present in sediment cores from each sample collection. In general, our observations were consistent with the spatial pattern of colonization described by Diaz et al. (2008). At Station BH03, near the sludge disposal area, dense mats of *Ampelisca* spp. were present in sediment cores beginning very early in the study and for most of the pre-diversion years, but more sparse to sometimes absent during the post-diversion period. Similarly, cores from Station BH08A, near the Nut Island outfalls, contained *Ampelisca* spp. mats

for most of the study years, but appeared to peak in density somewhat later than at Station BH03. *Leptocheirus pinguis* was also present at this site late in Period IV (2008). In contrast, *Ampelisca* spp. mats were absent in cores from Station BH02, the reworking site, for most years of the study; however *Leptocheirus pinguis* colonized this site late in Period IV (2007 and 2008), and was followed by *Ampelisca* spp. Amphipod mats were never present in cores from Station QB01, the least impacted site, although *Leptocheirus pinguis* was present in 2008 and early 2009.

2.6. Data analysis

Statistical analyses were kept to the most conservative approaches because assumptions of many of the more powerful approaches were not met by our data. Boston Harbor has been affected by human activities for centuries, so we did not have data from a pre-disturbance period, nor was there a control or reference site available within Boston Harbor. In addition, we had an unbalanced design, with 2 sites studied during 4 treatment periods and 2 sites studied during 3 periods, as well as unequal number of samples within periods. Treatment changes were implemented incrementally and, depending upon location, each station was impacted to a different degree by the loading changes. Finally, colonization by infauna differed at each station and was not directly quantified in this study.

Therefore, we used non-parametric techniques to evaluate trends in the time series data. After testing for the presence of autocorrelation and partial autocorrelation, we applied the Mann–Kendall test on untransformed data (Helsel and Hirsch, 2002; McLeod, 2011), either annual grand or individual station means of SOD, nutrient fluxes, denitrification, and sediment TOC by year. The Kendall tau (τ) statistic and p-value are given for results that indicated a significant trend. Trend lines are plotted using Sen-Theil estimates of slope and intercept (Bronaugh and Werner, 2013).

To describe site differences, and to facilitate cross-reference to other studies (Diaz et al., 2008; Taylor, 2010; Taylor et al., 2011), data for each site were averaged according to time periods corresponding to treatment changes (Fig. 2), These periods roughly match those previously defined by Taylor (2010) (Table 1), but exclude Period I because we have limited data from that period. We divided Period II to emphasize the changes in TSS loading that occurred with the upgrade from primary to secondary treatment (Fig. 2), which were of particular importance for the benthos, and because the addition of the two stations in the southern harbor occurred mid-period. Individual station means and standard errors were calculated for each period.

To explore the underlying drivers associated with observed changes in benthic fluxes, we first used correlation analysis to identify the most important explanatory variables. Subsequent analyses used simple linear regression, with R^2 and p-values for the regression given to describe the strength of relationships. All statistical analyses were performed using the statistical package R version 3.0.1 (R Core Team, 2013) or in Excel (Microsoft Office 2007).

3. Results and discussion

3.1. Overview of sediment–water fluxes

Over the timeframe of loading reductions examined by this study, annual sediment oxygen demand (SOD) averaged across all stations showed a significant decline, despite interannual variability (Fig. 3a). In the early phases of the harbor cleanup, SOD was quite high and variable (Fig. 3a and Supp. Fig. S2), reaching levels among the highest reported in the literature (Nixon, 1981; Forja et al., 1994). As loading reductions continued to be phased

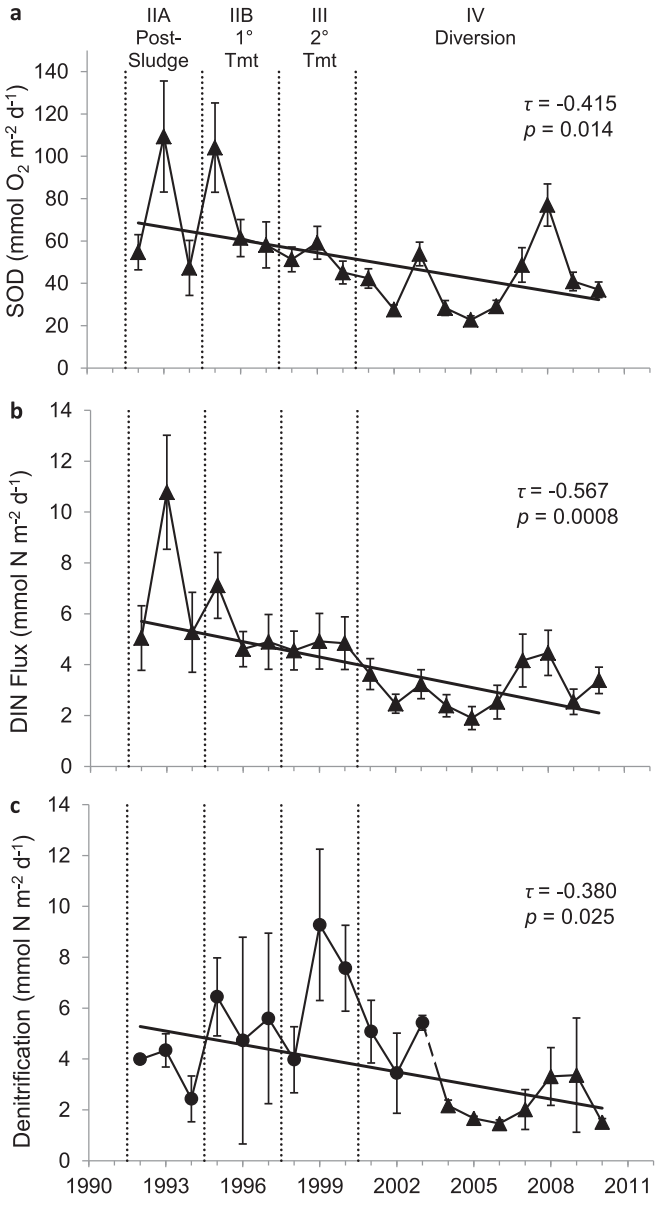

Fig. 3. Annual means across all stations (±SE) plotted by year for (a) S.O.D., (b) DIN efflux, and (c) denitrification. The heavy solid line is the Mann–Kendall trend line. Treatment periods are demarcated by dotted vertical lines (1° Tmt = full primary treatment; 2° Tmt = full secondary treatment). The circle symbols in panel (c) denote that denitrification data until 2004 are from the two northern harbor stations only.

in, rates and variability declined, such that by the end of the study they were comparable to unimpacted sites in similar embayments in the region (Banta et al., 1995; Fulweiler et al., 2010). Period average SOD decreased from 73 mmol m^{-2} d^{-1} in Periods IIA and IIB to 41 mmol m^{-2} d^{-1} in the post diversion period (IV) (Fig. 3a). Although harbor average rates and variability were lowest during the diversion period (IV), there were some noteworthy excursions in SOD that occurred near the end of the period associated with changes in the infaunal community at 2 stations (see Section 3.3 and Fig. S2).

In concert with SOD, average effluxes of dissolved inorganic nitrogen (DIN) were also highest during the early phases of cleanup (Period II) and declined significantly over time as loading reductions progressed (Fig. 3b). The two northern harbor stations decreased a total of 57% from the Period IIA average of

7.4 mmol m^{-2} d^{-1}. For all four stations, DIN flux decreased from 5.5 mmol m^{-2} d^{-1} during Period IIB to 3.1 mmol m^{-2} d^{-1} during Period IV, a 44% decrease. As noted for SOD, an infauna-associated increase in DIN fluxes was observed late in Period IV.

Denitrification followed a different trajectory than respiration and DIN fluxes, showing peaks in rates during Period III, following those for SOD and DIN, and then declining. Controls on sediment nitrification–denitrification, especially sediment redox conditions, may be strongly affected by bioirrigation (see Section 3.6) and therefore less directly responsive to loading changes than SOD or DIN fluxes. Even with the high rates occurring mid-study, the decline for the whole study timeframe was significant (Fig. 3c). For Period III, the average rate of denitrification was 6.9 mmol N m^{-2} d^{-1}, more than double the rate of Period IIA and on the high end of rates compiled for a range of aquatic sediments (Fennel et al., 2009). In Period IV, average denitrification decreased to 2.5 mmol N m^{-2} d^{-1}, very near the mean of the cited range, and, like SOD and DIN fluxes, included a temporary increase in rates near the end of the study.

There was no significant trend in annual average fluxes of dissolved inorganic phosphorus (DIP) over time (data not shown). With the exception of one year during Period IIA, DIP fluxes were small, <0.5 mmol m^{-2} d^{-1}. Small phosphate fluxes, especially early in the study, were somewhat unexpected given the eutrophic status of Boston Harbor (Conley et al., 2002), and were more similar to findings from less impacted sites (Forja et al., 1994; Fulweiler et al., 2010). Treatment period averages showed some site-specific differences, discussed below (see Section 3.3).

Fluxes of dissolved silicate (dSi) were always sizable and often both spatially and temporally variable, so although there was a downward trend in average annual fluxes over time, it was not significant (data not shown). Largest silicate fluxes with highest variability occurred at the beginning of the study, with an average for Period IIA of 11.4 mol m^{-2} d^{-1}. The average for the post-diversion period (IV) was 6.1 mol m^{-2} d^{-1}, but the period included years with high rates; for example, an excursion of over 10 mmol m^{-2} d^{-1} concurrent with increases in SOD, DIN efflux, and denitrification and the presence of infauna. Since silicate fluxes are largely controlled by dissolution and transport processes that are enhanced by bioirrigation (Berelson et al., 1998; Aller, 2001), high rates and variability of silicate flux in Boston Harbor may reasonably be attributed to patchiness in infaunal presence and abundance.

3.2. Sediment properties

Sediment organic matter content decreased over time at three of the four stations (Table 3), but changes were temporally and spatially variable. Largest reductions occurred at BH03, near the sludge disposal site, where mean TOC decreased from about 3.7% C during the post-sludge and full primary periods (IIA and B) to 2.1% C after outfall diversion (Period IV). A similar decrease was observed at Station BH08A, near the Nut Island outfall, where average TOC decreased from 3.3% for the full primary period (IIB) to 1.9% in Period IV. At Station QB01, most of the decrease in TOC occurred between the full primary (IIB) and full secondary (III) periods. Interannual variability at Station BH02 during the pre-diversion periods was larger than at the other three stations, likely due to sediment reworking. Consequently, there was no statistically significant change at this station, even though after diversion, TOC values at BH02 were always lower than in early periods and variability had decreased substantially. Overall, mean TOC was lower and less variable during the post-diversion period (IV), with period averages for the four sites falling in a narrowed range of 1.9%–2.3%.

Table 3
Changes in total organic carbon (TOC) content in the top 2 cm of sediment according to treatment period. Data are means (±SE) for each period. Significant decreasing trends with time were found for Stations BH03, BH08A, and QB01. (na = not available; ns = not significant).

	Period	Years	Station BH02	Station BH03	Station BH08A	Station QB01
TOC (%dwt)	IIA	1992–1994	1.9 ± 0.3	3.7 ± 0.1	na	na
	IIB	1995–1997	2.5 ± 0.3	3.8 ± 0.1	3.3 ± 0.2	3.2 ± 0.1
	III	1998–2000	2.2 ± 0.2	2.8 ± 0.1	2.3 ± 0.0	2.4 ± 0.1
	IV	2001–2010	2.0 ± 0.0	2.1 ± 0.1	1.9 ± 0.1	2.3 ± 0.0
TOC by year	τ		ns	−0.793	−0.79	−0.386
	p value		ns	<0.0001	<0.0001	0.046

Decreases in TOC were related to reductions in sewage-POC inputs, but the strength of the relationship varied by site. At the two stations nearest outfalls, BH03 and BH08A, sediment TOC content was very strongly correlated with loading of POC. At these stations, POC loading could explain about 74% of TOC content (BH03: $R^2 = 0.74$, $p < 0.001$; BH08A: $R^2 = 0.73$, $p < 0.001$). The relationship was less strong, although significant, at QB01, explaining 45% of the pattern in TOC ($R^2 = 0.45$, $p = 0.004$). At Station BH02, where we conjecture that reworking produced high variability, the relationship between POC loading and TOC was not significant.

Sediment redox properties in the harbor were strongly influenced by infaunal population dynamics. In a previous paper, we reported a clear correspondence between sediment redox profiles and benthic infauna colonization at Stations BH02 and BH03 (Giblin et al., 1997). We continued to observe that relationship at these two stations as well as the two southern harbor stations. The effects of infaunal activity on sediment redox conditions may be appreciated in SPI images (Diaz, used with permission). These images contrast a year when *Ampelisca* spp. was present at mat densities at Station BH03 (=Diaz Station T03; Fig. 4a), which was typical of most pre-diversion years at this site, with a year when *Ampelisca* spp. was scarce (Fig. 4b). A similar contrast is shown for the effects of *Leptocheirus pinguis* at Station BH02 (=Diaz Station T02; Fig. 4c and d), which colonized this site late in the post-diversion period, showing tunnels and feeding voids created by this amphipod. At both stations, the SPI images show a dramatic deepening of the oxidized zone (lighter colored sediments) and apparent redox potential discontinuity (aRPD) when high densities of amphipods were present.

Consistent with the visual evidence, measurements of sediment redox potential (Eh) and porewater sulfides showed trends that varied spatially and temporally, corresponding to amphipod presence. Redox status changed quickly at Station BH03 (Fig. 5a), but slowly at Station BH02 (Fig. 5b), with Stations BH08A and QB01 showing intermediate responses (data not shown).

At Station BH03, despite having the highest levels of sediment carbon, sediments became less reducing very quickly after loading reductions began (Fig. 5a). Early colonization by amphipods corresponded with a rapid shift in Eh profiles towards more positive values (compare 1992 to 1993 in Fig. 5a), a deepening of the aRPD, and a decrease in sediment sulfides (Giblin et al., 1997). 1993 was the last year we found measurable concentrations of HS^- in cores from this station. Typical profiles after this time showed Eh values generally >0 for the top 10 cm or deeper and no measurable sulfides.

At Station BH02, where animals were typically less numerous, sediments remained strongly reducing during most of the pre-diversion periods, with Eh values indicative of sulfate reduction (~−200 mV) co-occurring with high concentrations of HS^- very near the sediment surface (data not shown). Surface films of sulfur-oxidizing bacteria were noted during some early sample

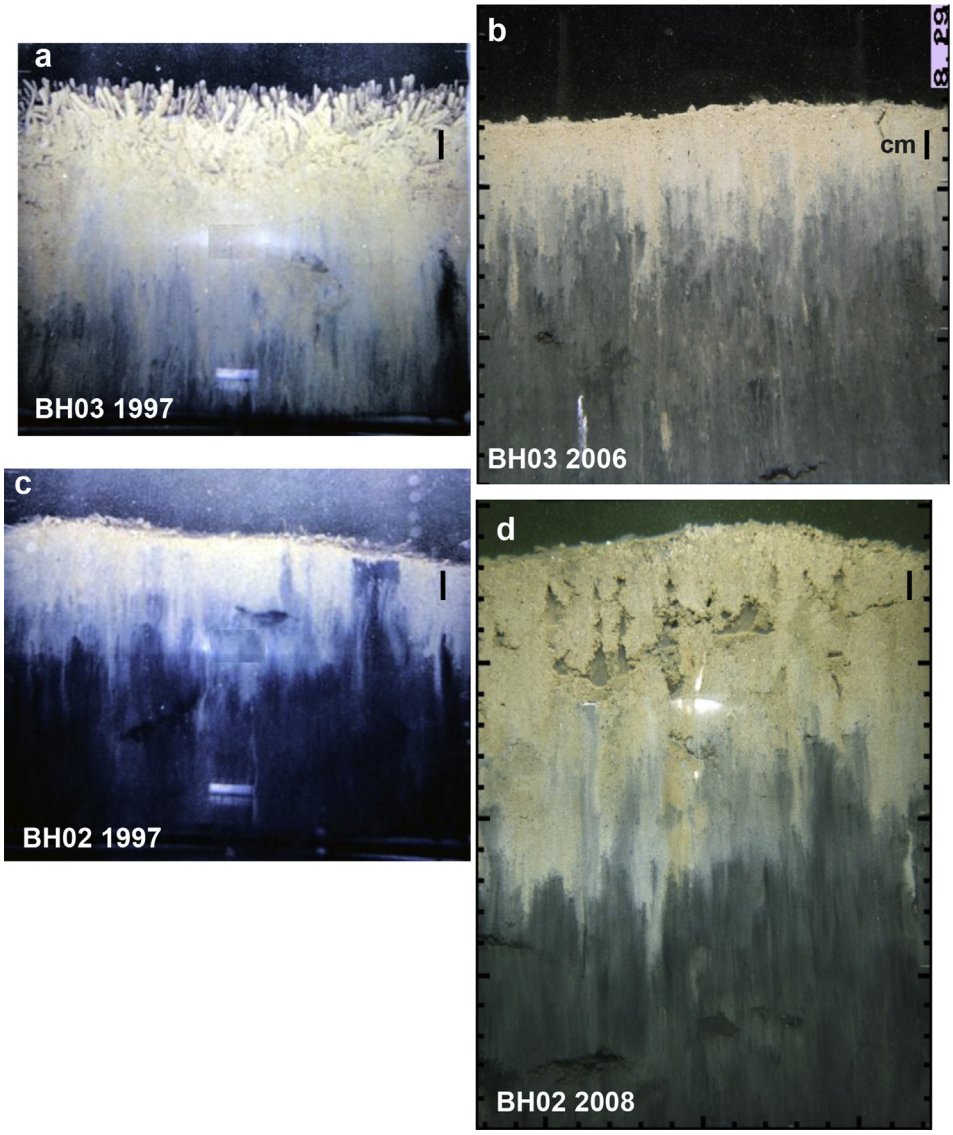

Fig. 4. Sediment profile images (SPI) showing deeper oxidized layers when amphipods were present: (a) Station BH03 in 1997; tubes of a dense *Ampelisca* spp. mat can be seen protruding from the sediment surface; (b) Station BH03 in 2006; *Ampelisca* spp. mat absent; (c) Station BH02 in 1997; amphipods absent; (d) Station BH02 in 2008; tunnels and feeding voids of *Leptocheirus pinguis* are visible. Images courtesy R. Diaz.

collections. HS^- remained detectible within a few cm of the sediment surface into the post-diversion period (IV) although there were episodic improvements in redox conditions during periods when we observed amphipod colonization (e.g. August 2001). Later into Period IV less reducing conditions became more sustained with more persistent colonization and by the end of the period, Eh profiles at times reflected highly oxidizing conditions produced by extensive bioirrigation (e.g. in 2008, Fig. 5b).

3.3. Spatial and temporal patterns in sediment fluxes

Within the overall trajectory of reduced benthic metabolism in the harbor, temporal patterns were site-specific and often appeared to be strongly influenced by infaunal population dynamics. These site differences offer insight into a range of responses in benthic metabolism that might be expected from OM reductions in other systems, particularly with respect to the effects of bioirrigation and bioturbation.

We did not measure bioirrigation directly, but it may be inferred from changes in the depth of the aRPD that were related to high abundances of amphipods. Annual measurements of aRPD made in August at two of our sites, BH02 and BH03, by Diaz et al. (2008) and Maciolek et al. (2011), were strongly related to summer (July–August) rates of SOD and to our visual assessments of amphipod presence in sediment cores (Fig. 6). As described above, Station BH03 was heavily colonized by *Ampelisca* spp. during most of the early years of the study when TOC content of the sediment was high. During these years the aRPD deepened and SOD was very high (Fig. 6a) In later year, when *Ampelisca* spp. was less abundant or even absent from the site, and as carbon content declined, aRPD and SOD declined. In contrast, at Station BH02 the aRPD remained shallow for most years (Fig. 6b). It deepened and SOD increased when *Ampelisca* spp. was present, but most markedly late in the post-diversion period IV (eg. 2008) when *Leptocheirus pinguis* colonized the site. Neither measure increased to the extent observed early at BH03, however, likely because sediment TOC had become relatively depleted by the end of Period IV.

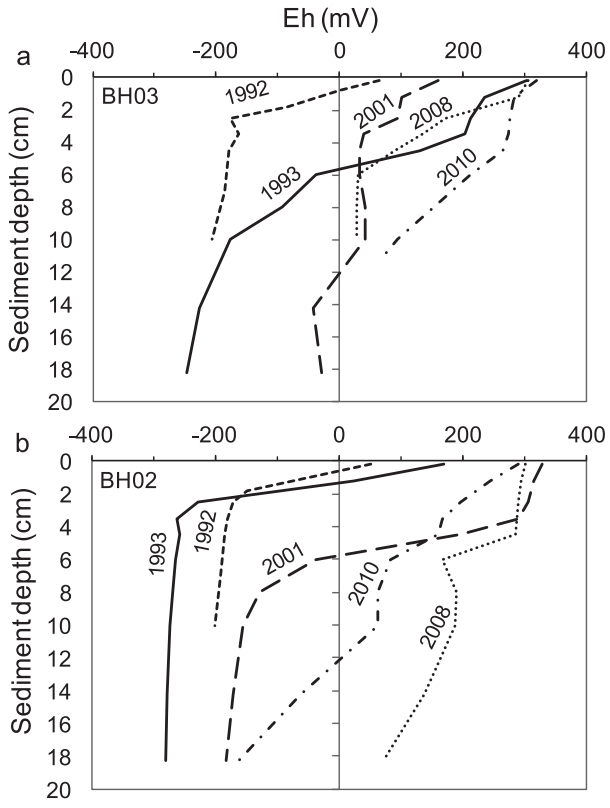

Fig. 5. Representative Eh profiles at (a) Station BH03 and (b) Station BH02 during August, when warmest temperatures of the season would predict most reducing conditions. Years shown are 1992: the first year of the study; 1993: appearance of *Ampelisca* spp. mats at BH03; 2001: the first year after outfall diversion; 2008: appearance of *Leptocheirus pinguis* at Station BH02; 2010: the last year of the study.

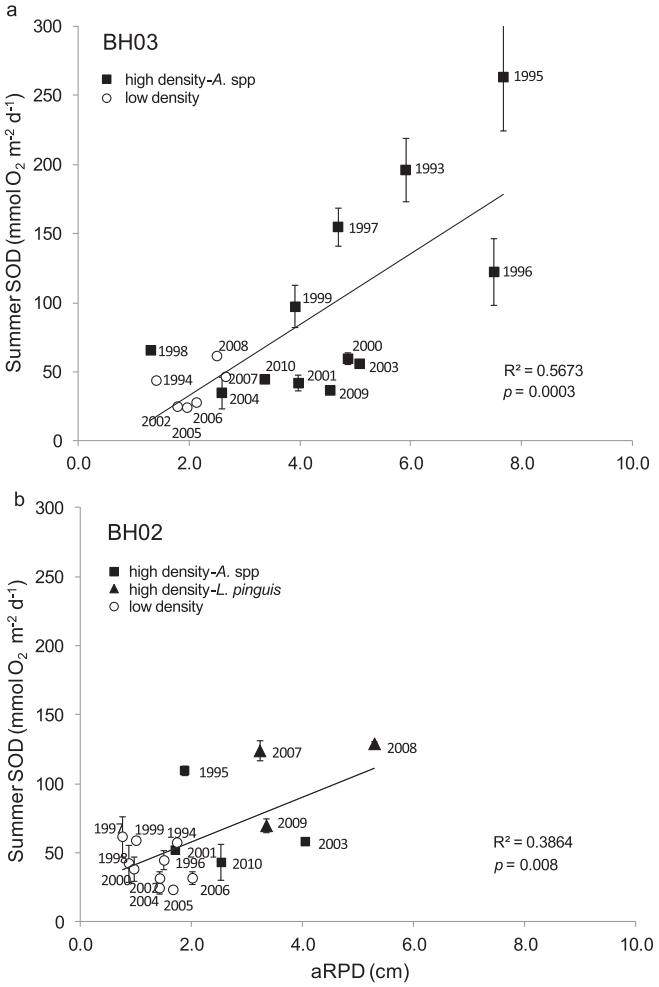

Fig. 6. Relationship of summer SOD to depth of the aRPD (Diaz, pers. com). Symbols indicate visual assessments of amphipod densities as observed in sediment cores for June and July of each year. (High density = amphipod tube mats or numerous tubes or holes noted; low density = few to no tubes or holes).

Sediment oxygen demand (Fig. 7a) showed largest changes at Station BH03, where sediments had received loading from sludge disposal and where infaunal activity was high. The decrease in annual averages over the 19 year time series was highly significant ($\tau = -0.509$, $p = 0.003$). Highest station average SOD (129 mmol m^{-2} d^{-1}) for a treatment period occurred during the full primary period (IIB), concurrent with highest TOC content and densities of *Ampelisca* spp. (Fig. 2b). By the next period, SOD at this site had decreased by about half, and by half again after diversion (IV). High respiration rates were also observed at Station BH08A in the southern harbor, the other station where the *Ampelisca* spp. mat was typically present, and showed a similar large decrease between periods III and IV concurrent with the decline of the mats. The overall change with time was also significant at this site ($\tau = -0.45$, $p = 0.017$). Less change was observed at Station BH02, where the sediments were reworked by tidal action, amphipod presence was inconsistent, and sediment Eh changed more slowly. At Station QB01, the least impacted site, oxygen uptake was lowest and least variable and did not change significantly.

For the first several years of the post-diversion period (IV), rates and variability of SOD were much lower than at the beginning of the study and the four stations seemed to have converged on a new "baseline". Prior to 2007, the post-diversion average for SOD was 34 mmol m^{-2} d^{-1}, less than half that of Period IIB In 2008, however, seasonal averages at Stations BH02 and BH08A increased to 106 and 102 mmol m^{-2} d^{-1}, respectively, following colonization by *Leptocheirus pinguis* in 2007 and 2008 and the return of *Ampelisca* spp. starting in 2008 (Fig. 3a and Fig. S2). An increase in rates was observed even at Station QB01, which had its highest seasonal average over the entire study, coinciding with a short-lived appearance of amphipods at this site. After this excursion, SOD returned to rates similar to the beginning of the period, and the four-station average for 2009–2010 was 39 mmol m^{-2} d^{-1}. In spite of higher respiration rates during 2007–2008, sediments had more positive Eh values (Fig. 5) showing that this increase in respiration could not be explained simply by an increase in organic matter deposition.

There is an extensive literature describing the effects of macrofauna on sediment biogeochemistry (for a review see Kristensen and Kostka, 2005), and it was clear that amphipod populations played a major role in mediating rates of exchange at the sediment–water interface in Boston Harbor. Partitioning of total benthic respiration into microbial and macrofaunal-enhanced components has suggested that macrofauna increase SOD by 2–3.5 times (Pelegri et al., 1994; Kristensen, 2000; Laverock et al., 2011). In a combination of *in situ* and laboratory experiments, Glud et al. (2003) found that up to 40% of the increase was direct faunal respiration, with the rest attributed to enhanced microbial respiration. An estimate of maximal effects within our observations was consistent with these studies. We comparedaverage SOD at station BH03for the summers of 1992, before amphipod colonization, and 1993, when rates peaked at this station and amphipods

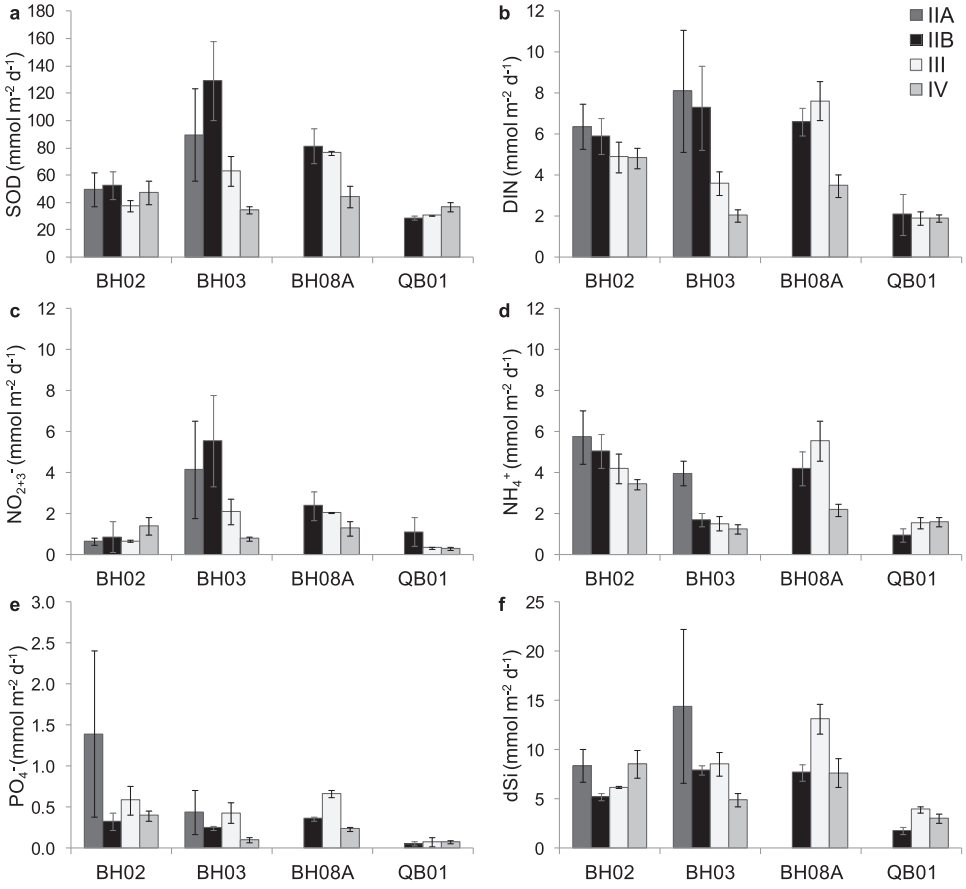

Fig. 7. Treatment period means (±SE) by station for (a) S.O.D., (b) DIN efflux, (c) NO_{2+3}^- efflux, (d) NH_4^+ efflux, (e) PO_4^- efflux, and (f) dissolved silica efflux.

were present in mat densities. Under these conditions, with sediment TOC similarly high and temperatures warm (averaging 4.1% and 3.7% TOC and 16°and 18°, respectively), we found a 3.2-fold increase in SOD after colonization.

Like SOD, DIN effluxes (Fig. 7b) showed the most change at Station BH03, with period average rates falling from 8.1 to 2.0 mmol m^{-2} d^{-1}, and most of the decrease occurring after the full primary period (IIB). A significant decrease was also observed at Station BH08A, despite an increase during the full secondary period (III). At both BH03 and BH08A, the declining trend in DIN flux was significant ($\tau = -0.567$ and -0.45, $p = 0.0008$ and 0.017, respectively). Although there was a decreasing trend in DIN fluxes at Station BH02, it was not significant. No change was observed at Station QB01, where DIN fluxes were generally smaller than at the other three stations, averaging about 2 mmol m^{-2} d^{-1} during all periods.

In their compilation of sediment nitrogen fluxes from a range of aquatic environments, Fennel et al. (2009) found that on average, DIN effluxes were primarily comprised of NH_4^+, whereas NO_{2+3}^- effluxes were often negligible. Departures from this general case, that is, when NO_{2+3}^- fluxes become important, are indicative of oxidized sediment environments, favorable for nitrification. The proportions of NO_{2+3}^- and NH_4^+ comprising the DIN flux from harbor sediments were site-specific (Fig. 7c and d) and changed over time. At Station BH03, both NO_{2+3}^- and NH_4^+ fluxes decreased over the period of study ($\tau = -0.626$, $p = 0.0002$ for NO_{2+3}^-, $\tau = -0.333$, $p = 0.05$ for NH_4^+ flux), but NO_{2+3}^- fluxes increased to levels well above those at the other sites before declining. This pattern is consistent with the presence of tube-building amphipods which have been shown to promote nitrification through bioirrigation, largely by increasing oxygen penetration into sediments (Mayer et al., 1995; Aller, 2001). At this station, over half of the DIN flux was comprised by NO_{2+3}^- until Period IV, when it dropped to 38% (compare Fig. 7c to d). At Station BH02, where bioirrigation was less important, NH_4^+ comprised over 85% of the DIN flux until Period IV. There was, however, a significant decline in NH_4^+ fluxes ($\tau = -0.386$, $p = 0.028$), contributing to a decrease in its contribution to DIN fluxes during Period IV to 71%. There was no significant change in NO_{2+3}^- fluxes at Station BH02, although there was an increase during Period IV related to the 2007–2008 colonization and oxidation of sediments by *Leptocheirus pinguis*.

In the southern harbor, NH_4^+ was typically the larger component of the combined flux. At BH08A, NH_4^+ fluxes showed a transitional increase but an overall decline ($\tau = -0.417$, $p = 0.027$), while NO_{2+3}^- fluxes showed a unidirectional though insignificant decline. NO_{2+3}^- fluxes at QB01 did drop significantly ($\tau = -0.45$, $p = 0.017$), but an apparent increase in NH_4^+ flux was not significant.

Release of dissolved inorganic phosphate (DIP) from sediments may also reflect sediment redox status, with larger fluxes associated with more reducing conditions (Conley et al., 2002). Accordingly, large effluxes of DIP occurred very early at Station BH02 (Fig. 7e), when sediments at this site were still strongly reducing and organic-rich. These large fluxes resulted in the high average DIP flux of 1.4 mmol m^{-2} d^{-1} for Station BH02 during Period IIA, accompanied by large variability. Otherwise, DIP release from harbor sediments (Fig. 7e) was often small, and in contrast to the other fluxes, tended to be lower when animal presence was high, for example, during Period IIB. This pattern was consistent with observations showing that under more oxic conditions such as are produced by bioirrigation, porewater DIP is scavenged by iron

oxides and sediments act as a phosphorus sink (Conley et al., 2002). An overall downward trend in DIP fluxes was observed at Stations BH02, BH03, and BH08A, but was statistically significant only at Station BH08A ($\tau = -0.5$, $p = 0.008$). DIP fluxes at Station QB01 were always very small and did not change. The overall average for the final period (IV) was 0.2 mmol m^{-2} d^{-1}.

Average fluxes of dissolved silicate (Fig. 7f) were always substantial. Although fluxes varied by period at all stations, a significant change over time was seen only at Station BH03 ($\tau = -0.412$, $p = 0.019$). As amphipods colonized this site during Period IIA, when sediments were very organic-rich, dSi fluxes became very high and variable, likely due to enhanced silica dissolution and porewater flushing (Aller, 2001). Fluxes averaged 14 mmol m^{-2} d^{-1} during this period, but decreased to 5 mmol m^{-2} d^{-1} by the end of the study (Period IV). At Stations BH02 and BH08A, dSi fluxes typically fell within the range seen at Station BH03, although an increase was observed at Station BH02 near the end of Period IV, when *Leptocheirus pinguis* "bloomed" and sediments at this site became deeply irrigated. Silicate fluxes at Station QB01 were always lower than at the other three sites.

Denitrification also showed substantial variability between sites over time (Fig. 8a). Others have demonstrated that denitrification may be enhanced when macrofauna are present, largely through enhanced nitrification (Mayer et al., 1995; Laverock et al., 2011). For example, in microcosm experiments, the amphipod *Corophium volutator*, with a u-shaped burrow similar to that of *Leptocheirus pinguis*, increased denitrification of NO_3^- produced within the sediments by 3-fold (Pelegri et al., 1994). It is consistent, therefore, that high average rates of denitrification at Station BH03 in periods IIB and III, driven by elevated summer rates, coincided with the periods of most active amphipod communities. Single time-point measurements reached 15.9 mmol N m^{-2} d^{-1} in August, 1999, at this site (Fig. S1). Similarly, Station BH02 had the highest average rate of the post-diversion period (IV), driven by elevated rates in summers of 2008 and 2009 (Fig. S1) when animal densities became high at this site.

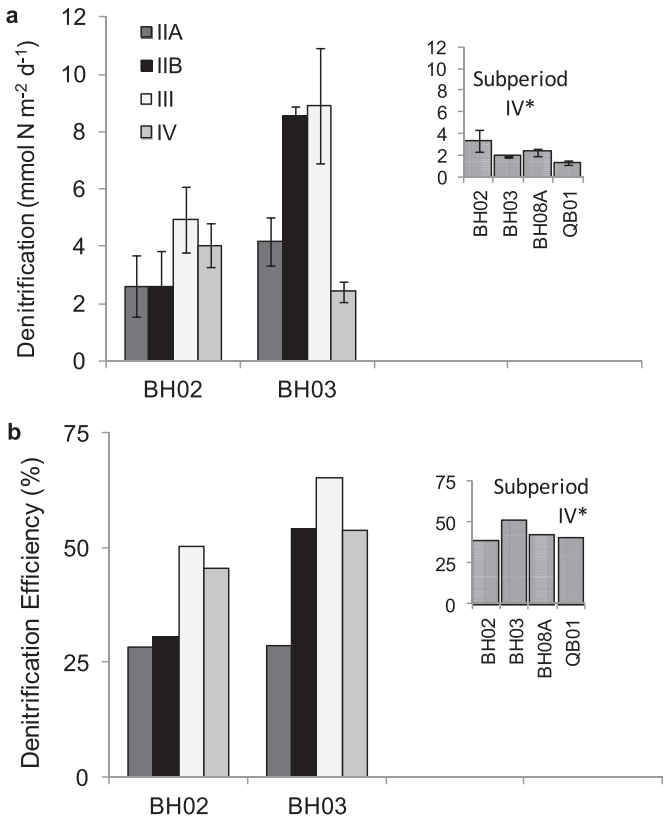

Fig. 8. Treatment period means (±SE) by station for (a) denitrification and (b) denitrification efficiency. Note that until 2004, denitrification was measured at Stations BH02 and BH03 only. The insets show data from all four stations during 2004–2010 (labeled Subperiod IV*).

Additional data from the two southern harbor stations, which became available during Period IV, are shown with temporally comparable (2004–2010) data for the two northern harbor stations (Fig. 8a inset, labeled Subperiod IV*). Rates at BH08A fell between those at BH02 and BH03, while rates at QB01 were somewhat lower. The average for all four stations was 2.2 mmol N m^{-2} d^{-1}.

Denitrification efficiency (DE), the fraction of the total inorganic N efflux that is lost via denitrification [DE = denitrification/(denitrification + DIN efflux)*100], increased through the pre-diversion periods (Fig. 8b). Often used as an index of the importance of nitrogen removal compared to nitrogen recycling (Berelson et al., 1998), DE was lowest in Period IIA (<30%), increased to a maximum in Period III (~60%) and declined again in Period IV (~45%). Highest efficiencies were reached at Station BH03, reaching 65% in Period III, and were likely related to animal effects (Section 3.6). Denitrification efficiency ranged between about 30% and 50% at the other three stations (Fig. 8b and b inset).

3.4. Contribution of sediment nutrient release to pelagic primary production

In many coastal systems, benthic recycling provides a significant percentage of nutrients required by pelagic primary production (Nixon, 1981). Very high rates of primary production were measured for the harbor during Period IIB, averaging 178 mmolC m^{-2} d^{-1} (Taylor et al., 2011). During this period, fluxes from the sediments could support about 20% and 15% of phytoplankton N and P demands, as estimated from Redfield ratios. After effluent diversion, primary production in Boston Harbor decreased nearly 50%, responding to reductions in water column concentrations of DIN and DIP of 56% and 40%, respectively (Taylor et al., 2011). Release of nutrients from sediments in Boston Harbor had also decreased, so we examined whether the relative contribution of nutrients supplied to the water column had changed. We found that the potential contribution from benthic recycling had increased somewhat for N to (27%), and had nearly doubled for P (to 28%). Moreover, the ratio of these autochthonous inputs to allochthonous loadings had changed substantially between the two periods. In Period IIB, DIN and DIP fluxes from sediments were equivalent to about 32% and 24% of TN and TP loadings (Taylor, 2010), respectively. After diversion (Period IV), DIN fluxes were equivalent to TN loading, and DIP fluxes were over twice loading inputs.

3.5. Organic matter controls on benthic metabolism

Loading of particulate organic carbon (POC), which contributed about 74%, 43%, and 38% of TSS loading (Fig. 2a) during Periods II, III, and IV, respectively, declined overall from 92.5 to 5 t d^{-1} (Taylor, 2010). In general, as POC loadings decreased, SOD and nutrient fluxes also decreased. Annual POC loading explained about a third of the variability in annual average SOD (all stations; $R^2 = 0.35$, $p = 0.012$) and half of the variability in average DIN fluxes ($R^2 = 0.53$, $p < 0.001$). Some water column responses in Boston Harbor lagged loading changes by as much as 5 years (Taylor et al., 2011), so we investigated whether a similar lag was present in the benthic response. We found the strongest relationships with a two-year lag (for SOD, $R^2 = 0.48$, $p = 0.001$; for DIN, $R^2 = 0.69$, $p < 0.001$].

This time scale is consistent with Berner's (1980) multi-G model for decomposition of organic matter of mixed reactivity and fits with the steep downward trajectory of initial loading changes, during which "fresh" loading would combine with the legacy of previous years. A similar phenomenon was observed at some sites in Chesapeake Bay that receive terrestrial inputs of nutrients and carbon (Brady et al., 2013). The investigators found that unexplained variability in SOD was correlated with the previous year's TN loading, and suggested a surplus of G_2 carbon was being utilized in subsequent years.

Decreases in SOD were also related to sediment TOC content, which was somewhat surprising since bulk TOC is often regarded as "worked-over" recalcitrant carbon and therefore a poor predictor of benthic metabolism (Kelly and Nixon, 1984; Banta et al., 1995; Hopkinson et al., 2001). As noted above, however, at three stations we found a significant correlation between sediment TOC and inputs of sewage POC, a relatively labile carbon source. Because we had TOC data to pair directly with SOD data for each station, we explored this relationship for site differences.

At Stations BH03 and BH08A (Fig. 9a), sediment TOC was strongly associated with SOD (for BH03, $R^2 = 0.48$, $p = 0.001$; for BH08A, $R^2 = 0.43$, $p = 0.006$). These sites showed the best correlation with POC inputs and were where amphipod communities were typically important. Carbon mineralization at these sites was likely facilitated by bioirrigation, which supports co-metabolism of carbon stores and transports oxygen and nitrate, strong oxidants, to anoxic zones in the sediment (Kristensen, 2000; Aller, 2001). Diaz et al. (2008) estimated that *Ampelisca* spp. could be responsible for 7%–18% of carbon reductions in Boston Harbor through direct consumption or by enhancing remineralization. In contrast, at Stations BH02 and QB01, which usually lacked amphipods, we found the more expected absence of relationship between sediment TOC and SOD (Fig. 9b).

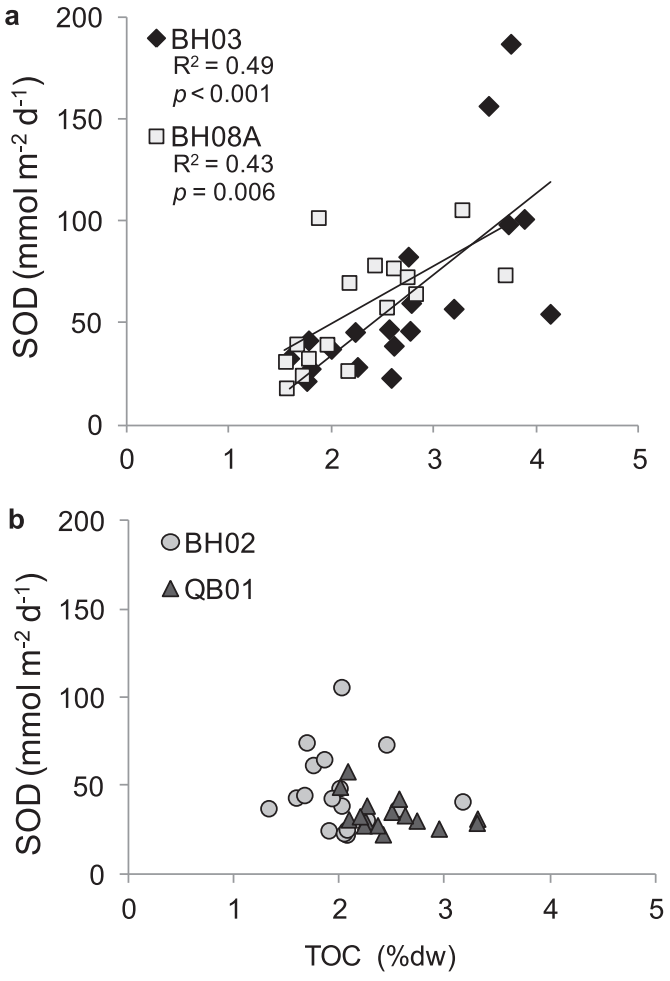

Fig. 9. Relationship of SOD (annual May–Oct averages) to organic carbon content of sediments at Stations (a) BH03 (1992–2010) and BH08A (1995–2010) where bioirrigation was important compared to (b) Stations BH02 (1993–2010) and QB01 (1995–2010) where bioirrigation was typically not important.

3.6. Denitrification

By transforming inorganic forms of nitrogen to N_2 gas, denitrification renders some portion of DIN unavailable to most organisms and therefore acts as nitrogen sink (Seitzinger, 1988; Nixon et al., 1996). At the beginning of the Boston Harbor Project (end of Period I to Period IIA), Giblin et al. (1997) found that although rates of denitrification were substantial, the capacity of the sediments to mitigate the very high loading was relatively small. At that time, denitrification could account for only about 17% of the TN loads to the harbor, much less than found for many coastal systems (Nixon et al., 1996). Burial was estimated to account for about 2% of inputs, leaving the majority of inputs being exported to Massachusetts Bay. Our assessment for Period IV indicated that denitrification became a more important N sink after most inputs were removed by diversion, even though rates had declined. Using loading estimates for Period IV (Taylor, 2010) and assuming no change to burial, we found that denitrification could account for about 41% of current loading.

A study of denitrification conducted during the early years of the Boston Harbor Project found that denitrification was linearly related to TOC content of surficial sediments in Boston Harbor (Nowicki et al., 1997). The four stations sampled at that time had sediments that spanned a nearly 10-fold range of TOC. One endmember was Station BH03 with its organic-(sewage)-rich sediments (average TOC of 3.8%). The other endmember was a very sandy site with TOC of about 0.4%. In this case, the range of both content and lability allowed the bulk TOC to serve as a reasonable predictor of denitrification. We investigated whether this relationship would hold in the present study, which did not include the very low-carbon, sandy site, but which did include post-diversion years with relatively depleted carbon stores in the sediments. For the full dataset, there was a relationship between annual average TOC and denitrification, but TOC explained less than 20% of the observed variability ($R^2 = 0.19$, $p = 0.001$). Parsed by station, however, the relationship was significant only at BH03 and BH08A ($p = 0.006$ and 0.03, respectively), as was the case for the relationship with SOD. At these two sites, where nitrogen cycling was enhanced by bioirrigation, TOC could explain between about 35% and 65% of the observed variability ($R^2 = 0.36$ and 0.66, respectively).

A better predictor for denitrification in many systems is sediment oxygen demand. For example, Seitzinger and Giblin (1996) used SOD to develop a regression model for denitrification in continental shelf sediments. Similarly, Piehler and Smyth (2011) found strong linear relationships between SOD and denitrification across a range of inter- and sub-tidal habitats in coastal North Carolina. In Boston Harbor, denitrification was significantly related to SOD at three of the four stations (Fig. 10a–c). The slopes of the relationships were somewhat lower than found for continental shelves ($m = 0.116$; Seitzinger and Giblin, 1996), but similar to those found by Piehler and Smyth ($m = 0.04$–0.07; 2011) for shallow water habitats. These researchers suggested that higher slopes for the SOD and denitrification relationship are related to tight coupling between nitrification and denitrification. Such coupling

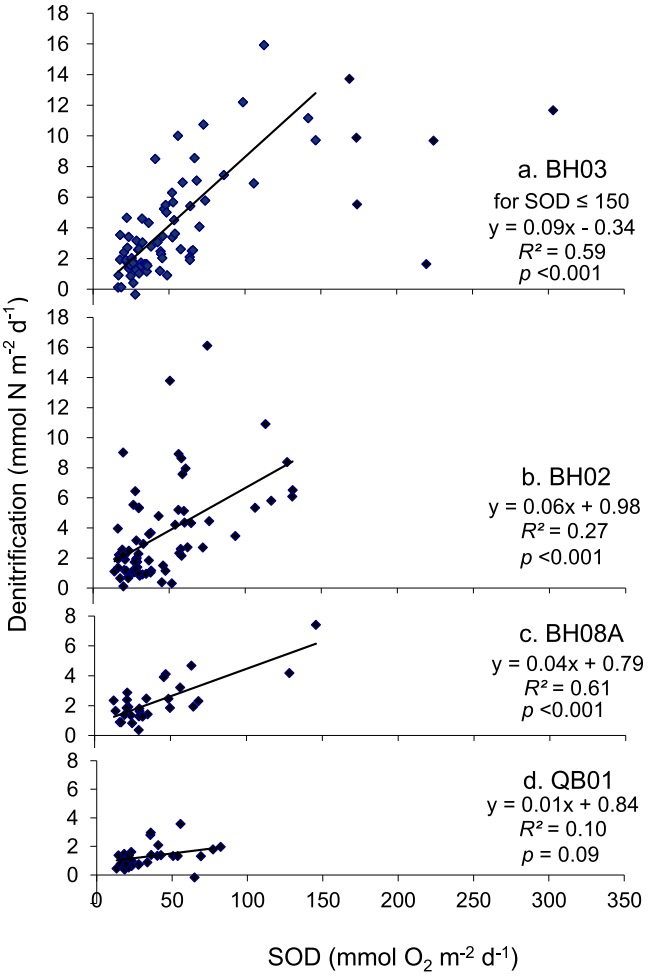

Fig. 10. Relationship of denitrification to sediment oxygen uptake at (a) Station BH03, (b) Station BH02, (c) Station BH08A, and (d) Station QB01 showing a linear response until very high rates of SOD are reached, as at Station BH03. The regression for Station BH03 includes only data where SOD $\leq$150 mmol m^{-2} d^{-1}, the apparent transition point above which the relationship became non-linear.

may in turn be related to redox gradients created by animal burrows (Aller, 2001), or in their case, "structured" habitats with roots and rhizomes. Our findings are consistent with this argument since the sites in Boston Harbor with higher slopes tended to be those with sediments structured by animals. At the site that did not show a significant relationship, Station QB01, denitrification rates were lower than at the other stations (Fig. 10d), and biogenic structure was minimal.

At Station BH03, where SOD reached rates over three times those in the studies above, the relationship between SOD and denitrification did not remain linear. At SOD higher than about 150 mmol m^{-2} d^{-1}, denitrification appeared to reach an asymptote or decline, decoupled from SOD. A curvilinear response is predicted by sediment modeling in the case where very high SOD is associated with reducing conditions that inhibit nitrification (DiToro, 2001) but at Station BH03, the efflux of nitrate suggests a different cause. Highest rates of SOD in Boston Harbor coincided with the presence of either the *Ampelisca* spp. mat colonies or *Leptocheirus pinguis* colonies, and we suggest that the loss of the linear relationship was related to the density of these animals, as was found by Braeckman et al. (2010). Although bioirrigation generally enhances nitrification and denitrification (Pelegri et al., 1994; Mayer et al., 1995), at extremely high burrow or tube densities and related porewater irrigation rates, transport rates of solutes out of porewaters may surpass reaction rates and thereby limit coupled nitrification and denitrification. Modeling exercises (Gilbert et al., 2003) described an optimum burrow density for denitrification, beyond which denitrification declined. A similar result was found using spacing between burrows (Michaud et al., 2010); denitrification efficiency increased as burrows became more tightly spaced up to an optimum spacing, and then declined sharply. This was likely the case in areas of Boston Harbor like BH03, where mat communities could contain on the order of 100,000 *Ampelisca* spp. per square meter and over twice that number that for total infauna (Kropp et al., 2000).

4. Conclusions

In Boston Harbor, sediment metabolism and nitrogen cycling responded quickly to reductions in loading of sewage solids and wastewater effluent. There have been few long-term studies of sediment responses to whole ecosystem reductions in nutrient loading for comparison. Studies from systems that have been examined (Smith et al., 1981; Arvai et al., 2002), together with experimental work (Kelly et al., 1985) and modeling (DiToro, 2001), support our findings that at least in relatively well-flushed systems, sediments may respond rapidly to loading reductions and may not retard ecosystem recovery for long periods of time.

The rapid response in Boston Harbor was likely enhanced by the relative absence of hypoxia that prevented the system-wide loss of local infaunal populations (Thrush et al., 2009). Therefore, when reductions to organic loading began, sediments were recolonized quickly (Diaz et al., 2008), and consequent bioirrigation accelerated the biogeochemical recovery of the sediments. The fact that oxic conditions were maintained in the water column also prevented feedback loops that occur with hypoxia/anoxia that intensify eutrophication (Conley et al., 2009). For example, nitrification, and therefore denitrification, was not inhibited, and phosphorus release was not enhanced (Diaz and Rosenberg, 2008; Kemp et al., 2009; Middelburg and Levin, 2009).

The trajectory of recovery, however, was not simple. In contrast to general expectations that reductions in loading should lead to decreases in SOD and nutrient fluxes (Kelly and Nixon, 1984), harbor sediments initially exhibited excursions of high oxygen consumption, DIN release, and denitrification related to colonization. These events continued to occur at individual sites through the end of our study [and will likely continue as patches of the benthic community progress through successional stages (Rosenberg, 2001)], but the magnitude of the excursions decreased. Overall, we observed a reduction of both the mean of the fluxes and their variability as organic matter content of the sediments declined and benthic communities matured (Diaz et al., 2008).

The changes in sediment processes documented here show that the benthic environment of Boston Harbor has improved substantially over the last two decades, with increased sediment oxidation and denitrification efficiency, as well as declining oxygen and nutrient fluxes. Our results, together with findings from water column (Taylor, 2010) and benthic infaunal (Diaz et al., 2008) studies, indicate that reductions in wastewater loadings of organic matter and nutrients have dramatically improved environmental conditions in the harbor.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2014.09.018.

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